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Effect of a Single Dose of T_3 on the Uptake of (2-^{14}C)-Ethanolamine into Chicken Liver Phospholipids

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The effect of a single dose of 3,3',5-triidothyronine (T_3) on the (2-^{14}C)-ethanolamine uptake into liver phosphatidylethanolamine (PE) and lysophosphatidylethanolamine (LPE) were determined in chicken over a period of 0.25 to 6 h after radioactive precursor injection. In all cases animals received the T_3 dose intraperitoneally 5 h before the injection of the labeled compound.

T_3 enhances the incorporation of (2-^{14}C)-ethanolamine into liver PE and LPE. The maximum uptake takes place at 0.25 h for LPE and 0.5 h for PE after the precursor administration. A great and significant hormone-dependent increase in the incorporation of labeled compound is observed in LPE. Lipid P associated to PE and LPE remains constant throughout the experiment, and does not vary with hormone treatment. It is suggested that T_3-injection increases, either directly or through other metabolic processes, PE and LPE turnover in chicken liver cells.

Key words: T_3, Phospholipids, Liver, 2-^{14}C-ethanolamine.

Extensive *in vivo* and *in vitro* studies by different authors, such as TREWHELLA and COLLINS (12) have demonstrated that phosphatidylethanolamine may be synthesized following the CDP-ethanolamine pathway by ethanolamine phosphotransferase. The effect of thyroid hormones on rat liver and lung phospholipids has been studied by different authors (5, 8). SANCHO *et al.* (11) have suggested that T_3 administration to newborn chicken increases the incorporation of inorganic ^{32}P into liver phospholipids; also they have observed a hormone-dependent activation of lipid metabolism. ARRONDO *et al.* (1) have shown that T_3 activates the enzymes

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involved in chick liver glycogen metabolism before the protein synthesis enhancement produced by the hormone.

The present work deals with the *in vivo* incorporation of (2-^{14}C)-ethanolamine into chicken liver phospholipids 5 h after T_3 hormone administration. Total lipid phosphorus associated to PE and LPE was also assayed throughout the experiment. The *in vivo* effect of a single T_3 dose on the PE pathway described by TREWHELLA and COLLINS (12) has been also studied.

Materials and Methods

The experimental animals were 3-day old chicken (*Gallus domesticus*). Newborn chicken were kept for two days in our laboratory and fed a standard diet with water *ad libitum*. After this time they were deprived of food for 3 1/2 h prior to T_3 administration. Chicken were divided into two groups, control and treated animals; control group was injected intraperitoneally with 0.2 ml saline solution; treated group received in the same way 255 ng T_3/g body weight in 0.2 ml saline. After 5 h, both groups received intraperitoneally 4.4 × 10^6 d.p.m. of (2-^{14}C)-ethanolamine 44 mCi/nmol in 0.2 ml saline. The labeled compound was purchased from Radiochemical Centre Amersham, England. Chicken were killed 0.25, 0.5, 1, 2, 3, 4 or 6 h after (2-^{14}C)-ethanolamine injection. Livers were immediately removed, weighed and frozen in liquid nitrogen.

Lipids were extracted by blending the livers with chloroform : methanol : hydrochloric acid 12.5 N (220 : 100 : 1) in a Potter-Elvenhjem homogenizer with a teflon pestle. 0.1 N hydrochloric acid was added and blended again. The mixture was centrifuged and aqueous phase removed by aspiration. The lipid phase was taken to dryness under vacuum and stored at —20° C until analyzed. All solvent and chemicals used were reagent grade. Phospholipids were separated by thin layer chromatography (TLC) on silica gel H according to FEDRIANI *et al.* (2). Phospholipid fractions were visualized by exposure to iodine. The different phospholipid classes were identified by their Rf and by comparison with genuine standards (Sigma) (2). Phosphorus determinations were perfomed according to FISKE and SUBBAROW (3), after previous digestion with perchloric acid. The percentage of assayed phospholipids was nearly to 80 % of the total lipidic phosphorus.

Phospholipid fractions were scraped into counting vials (7). The scintillation cocktail was 0.03 % w/v POPOP and 0.7 % w/v PPO in 1.4-dioxane containing 10 % w/v naphthalene and diluted with 0.2 volume of water. Phospholipid radioactivities were determined in a Nuclear Chicago Mark II liquid scintillation counter. Sample quenching was monitored with internal and external standards. Statistical significance was tested using the Sutdent's *t* test.

Results and Discussion

The incorporation of (2-^{14}C)-ethanolamine into liver PE is shown in figure 1a. In control animals, the uptake is maximum 0.5 h after injection of radioactive precursor, and then decreases gradually. In T_3-treated chicken the maximum of specific activity has already been obtained 0.25 h after radioactive precursor injection. 1 h later the profile of (2-^{14}C)-ethanolamine incorporation is similar in T_3-treated and control animals. It can be seen that T_3 has an important effect on the specific activity of PE between 5 and 5.5 h after T_3 administration; this can be due to an increase in the turnover of chick liver PE mediated by a single dose of the hormone. About 1 h after the precursor injection, the T_3-treated

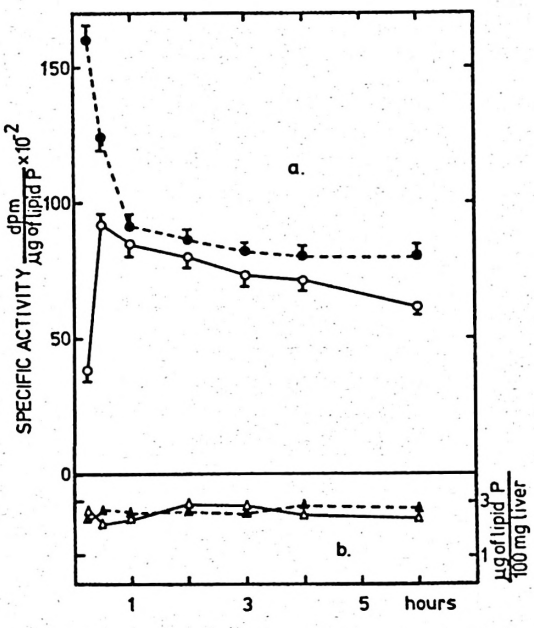

Fig. 1. *Time course of incorporation of (2-^{14}C)-ethanolamine into chick liver phosphatidylethanolamine (PE).*

a) The specific activities are expressed as dpm/µg lipid P ± S.E.M. (O) control; (●) T_3-treated. The injected dose of labeled compound at t = 0 is 4.4 × 10^6 dpm for each animal. When required, chicken received a T_3 dose 5 h before administration of the labeled compound. Each point represents an average of 5 values (mean ± S.E.M.). The differences between means have a P ≤ 0.001 for 1/4 - 1/2 h. The other values are not significant.

b) Lipidic phosphorus of liver phosphatidylethanolamine (PE) expressed as µg lipid P/100 mg liver ± S.E.M. (△) controls; (▲) T_3-treated. Treated chicken received T_3 dose 5 h before labeled compound administration. Differences between means are not significant.

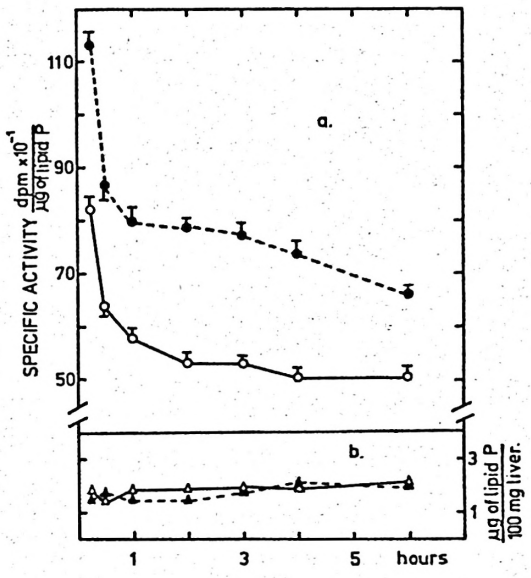

Fig. 2. *Time-course of incorporation of (2-^{14}C)-ethanolamine into chick liver lysophosphatidylethanolamine (LPE).*

Legends as fig. 1 except in a) where all differences between means have a P ≤ 0.001.

chicken have the same levels of specific activity than the controls for the PE. Total PC contents, measured as lipid P (figure 1b) do not vary with hormone injection nor as a funcion of time. Consequently, the uptake of labeled choline observed between 0.25 to 1 h in T_3-treated animals is due to an increase in label density of liver PE and not to an increase of total PE in liver; to sum up the T_3-hormone increases the turnover of this phospholipid.

Figure 2a depicts the incorporation of (2-^{14}C)-ethanolamine into liver LPE; a great incorporation is observed from the first 0.25 h. Differences between means are highly significant over all the time-course assayed; the T_3-hormone appears to act as an activator on the turnover of this phospholipid. Studies of total LPE content in liver phospholipids (fig. 2b) fail to show any differences between control and hormone-treated chicken, indicating again that the effect of T_3 is to increase phospholipid turnover, rather than net synthesis.

Similar effects of thyroid hormones have been described (4, 6, 9) in rat and rabbit lung cells, using (methyl-^{14}C)-methionine as a radioactive precursor. Other authors (10, 12) who have studied the incorporation of (2-^{14}C)-ethanolamine

into chicken liver phospholipids, have found that not all the molecular species of PE are methylated to PC and that, therefore, an important amount of (2-^{14}C)-ethanolamine remains linked to some molecular species of PE.

Our results suggest that the increase in (2-^{14}C)-ethanolamine uptake in T_3-treated chicken is due to a hormone-dependent activation of phospholipid turnover. However an interference by other endocrine and/or metabolic effects unavoidable in *in vivo* experimentation, cannot be discarded. Further *in vivo* and *in vitro* studies are being carried out at present.

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Resumen

Se determina el efecto de la hormona T_3 (triyodotironina) sobre la incorporación de la (2-C^{14})-etanolamina a fosfolípidos hepáticos de pollo, principalmente fosfatidiletanolamina (PE) y lisofosfatidiletanolamina (LPE), durante un período de tiempo de 0,25 a 6 h después de la inyección del compuesto radiactivo. Los animales reciben la dosis de T_3 por vía i.p. 5 h antes de la administración del precursor radiactivo. Un importante y significante efecto de la T_3 se observa en el caso de la LPE. El fósforo lipídico de PE y LPE permanece constante e inalterado por la hormona en todo el tiempo estudiado. Se sugiere que la inyección de T_3 aumenta el recambio del precursor radiactivo a dichos fosfolípidos ya sea directamente o bien mediante otros procesos metabólicos.

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